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Growth, rubber, and resin evaluation of two-year-old transgenic guayule

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Abstract

Guayule (*Parthenium argentatum* Gray) is a desert shrub that is a source of hypoallergenic, high-quality latex and rubber. Improvements in rubber content and yield have been made through conventional selection techniques. Further improvements are being attempted by transforming guayule with one of three genes encoding various allylic diphosphate synthases in the rubber biosynthesis pathway. The objective of this study was to evaluate the effect of these transgenes on growth, rubber and resin production, in field-grown guayule.

Tissue culture-generated transgenic plants of the lines AZ 101, AZ-2 and N6-5 were planted in two field plots in 2001 and 2002. In both plots, plant height and width were measured monthly. Branches from each plant were sampled every four months starting at one year of growth. Resin and rubber were quantified by gravimetric analysis after being sequentially extracted with acetone (resin) and cyclohexane (rubber). The 2001 plot was harvested at the end of two years of growth.

Transformation had no significant effect on growth of AZ-2 and N6-5 in the two years of the 2001 planting and the first year of the 2002 planting. In the 2001 planting, transformation appeared to have a drastic effect on the height and width of transformed AZ 101 compared with its empty vector control; however, the field in this study was not randomized and lacked non-transformed controls. In the 2002 planting, which was randomized and contained both positive and negative controls, the AZ 101 transformants were significantly larger than the empty vector AZ 101 control, but were not significantly different from the non-transformed controls.

In the 2001 planting, resin concentration increased throughout the year up to January 2003, but decreased by the time of harvest in March 2003. Rubber concentration, on the other hand, was high in May 2002, but decreased throughout the summer, before steadily increasing during the winter months.

Keywords: Guayule; Transgenes; Farnesyl pyrophosphate synthase (FPP); Geranylgeranyl pyrophosphate synthase (GGPP); Hexa-heptaprenyl pyrophosphate synthase (H-HPP); Resin; Rubber

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1. Introduction

Guayule (*Parthenium argentatum* Gray) is a shrub native to the Chihuahuan desert and is currently being investigated for cultivation in the arid southwestern United States as a source of high-quality, hypoallergenic latex and rubber (Thompson and Ray, 1989). The bulk of guayule rubber synthesis occurs during the winter months (Ji et al., 1993). Breeding efforts have been aimed at increasing the rubber content and overall yield with the most recent efforts focused on single-plant selections among polyploid apomictic plants and interspecific hybridization with other *Parthenium* species (Thompson and Ray, 1989). Breeding for increased rubber yield has been difficult because the most commonly used varieties of guayule are polyploid and reproduce apomictically at variable percentages (Thompson and Ray, 1989; Ray et al., 1990; Keys et al., 2002).

An alternative approach to increase rubber production is to target the rubber biosynthesis pathway directly using recombinant DNA technology (Cornish, 2001). The obvious and most desirable target is the rubber transferase gene, which catalyzes the addition of isopentenyl moieties from isopentenyl pyrophosphate (IPP) units to the rubber molecule (Backhaus et al., 1991; Cornish, 2001). However, the rubber transferase gene(s) have not yet been cloned, and therefore, other steps in the rubber synthesis pathway have been targeted.

An indirect approach to increase rubber production is to increase the amount of allylic pyrophosphates, which are initiators of rubber biosynthesis, available to the rubber transferase. This follows from in vitro experiments showing that the rate of rubber biosynthesis is dependent upon the concentration of the initiator (Tanaka et al., 1995, 1996; Tangpakdee and Tanaka, 1998; Castillon and Cornish, 1999; Cornish, 2001). However, allylic pyrophosphates are also precursors to numerous other compounds, such as chlorophyll and gibberellins that are essential for plant growth and development (Oh et al., 2000). Therefore, any transformation with genes for allylic pyrophosphate synthases could lead to multiple phenotypic effects and not just changes in rubber production.

In this report, we evaluate the effect of three different allylic pyrophosphate synthase transgenes placed into three guayule lines, on growth, resin and rubber production under field conditions.

2. Materials and methods

2.1. Generation of transgenic guayule lines

Three guayule lines, AZ 101, AZ-2, and N6-5, were used in the transformation. AZ 101 is an interspecific hybrid, with low rubber content but high biomass production. AZ-2 was selected for high biomass, but also has low rubber concentration. N6-5 in contrast was selected for high rubber concentration, yet has low biomass (Thompson and Ray, 1989; Ray et al., 1999). Each breeding line was transformed with at least one of three different genes using the *Agrobacterium*-mediated transformation (Pan et al., 1996). The genes, farnesyl pyrophosphate synthase (FPP) (Koyama et al., 1993), geranylgeranyl pyrophosphate synthase (GGPP) (Ohnuma et al., 1994) and a mutated form of geranylgeranyl pyrophosphate synthase, hexa-heptaprenyl pyrophosphate synthase (H-HPP) (Ohnuma et al., 1996), are all responsible for producing initiator molecules for rubber biosynthesis in the isoprenoid pathway. Each gene, under control of a constitutive promoter, was integrated into the guayule genome using a binary vector containing a neomycin phosphotransferase II marker gene (McBride and Summerfelt, 1990). The presence of the genes was confirmed by PCR (Katrina Cornish, personal communication). Positive controls were transformed with a binary vector containing the kanamycin selectable marker only. All transplants for both field plantings were generated from tissue culture and were grown in a greenhouse at the University of Arizona for three months before being transplanted into the field. The APHIS permit number for transgenic field release was 00-214-05n.

2.2. Field study for 2001

The 2001 field study was conducted at The University of Arizona Maricopa Agricultural Center, Maricopa, Arizona, from May 2001 to March 2003. A total of 130 plants were planted in the field, representing 21 different transformation events (FPP: AZ 101 $n = 5$, AZ-2 $n = 6$, N6-5 $n = 5$; GGPP: N6-5 $n = 16$; H-HPP:

AZ 101 $n = 3$, N6-5 $n = 68$) and six positive controls (AZ 101 $n = 20$, AZ-2 $n = 2$, N6-5 $n = 5$). All plants from the same transformation event were grouped together in the field without randomization. Transformants of the same variety were also grouped together within the field without randomization. The field was irrigated every 14 days from March to October and every 42 days from October to March.

Height and width were measured monthly starting in November 2001. Plant width was the average of two perpendicular width measurements per plant. In addition to height and width, secondary compound production was monitored throughout the second year of growth, from May 2002 through March 2003. One or two branches were removed from each plant within the plot in May, September and January, dried at 80 °C for two days and then ground in a coffee grinder. Resin and rubber concentration were determined using 0.5000 g samples by gravimetric analysis (Black et al., 1983) using acetone and cyclohexane, sequentially, to isolate resin and rubber, respectively. In March 2003, the above-ground biomass was harvested, run through a chipper, and dried at 80 °C for two days before the secondary compounds were extracted as described previously. Fresh weight was recorded at the time of harvest, and dry weight was calculated by subtracting the percent moisture of the chipped fresh weight and dry weight of a sample dried at 100 °C for two days. Total resin and rubber yields per plant were calculated from the percent resin or rubber and the dry weight of the plant.

2.3. Field study for 2002

The May 2002 field planting was planted adjacent to the May 2001 planting. The second field of transgenic plants contained 195 plants from seven different transformation events (FPP: AZ 101 $n = 37$, AZ-2 $n = 17$, and N6-5 $n = 17$; GGPP: N6-5 $n = 16$; H-HPP: N6-5 $n = 34$), with two negative and two positive controls, and was set up as a randomized complete block with four replications. Non-transformed AZ-2 ($n = 19$) and AZ 101 ($n = 20$) were included as negative controls, as well as AZ 101 ($n = 20$) and N6-5 ($n = 19$) empty vector positive controls. All other lines were the same as in the 2001 planting, with the exception of AZ 101 transformed with H-HPP, which was not available. Growth was measured as described for the 2001 planting.

2.4. Data analysis

All data from a line that contained the same gene were analyzed together, as there were no differences between transformation events as determined using linear regression (i.e., all 13 H-HPP transformations of N6-5 were analyzed as one data set). The data were analyzed using multiple regression in the fit model platform of JMP (Sall et al., 2001). Comparisons among transformants and among genes were done using Tukey's HSD. Comparisons between a transformant and its controls were done using orthogonal contrast. A P-value = 0.05 was used for testing significance.

3. Results

3.1. Growth

In the 2001 planting, all plants actively grew between April and October, with no active growth from October through March (Fig. 1). Due to the similar growth trends among all three lines, only the data set for AZ 101 is shown. No significant differences were observed in either height or width between N6-5 FPP, N6-5 GGPP, and N6-5 H-HPP and their corresponding empty vector control. No significant differences in growth were present between the AZ-2 FPP and AZ-2 empty vector control (data not shown). On the other hand, AZ 101 FPP and AZ 101 H-HPP were significantly taller and wider than the empty vector control (Figs. 1 and 2). The differences in width were significant from November 2001 through September 2002. By final harvest no significant difference existed in width between AZ 101 FPP and AZ 101 empty vector control (Fig. 2). Not only were there differences between AZ 101 FPP, AZ 101 H-HPP and AZ 101 empty vector control, but also all of the N6-5 and AZ-2 transformants were significantly shorter than AZ 101 FPP and AZ 101 H-HPP (data not shown). Differences in width between transformants were much more variable than differences in height, but no significant differences were observed among transformants at final harvest (data not shown).

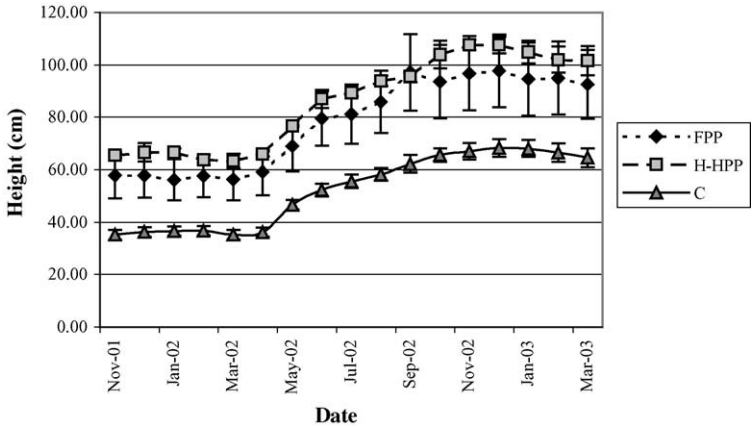

Fig. 1. Mean height (±S.E.) of transgenic guayule (AZ 101) from November 2001 through March 2003. The transgenes included are farnesyl pyrophosphate synthase (FPP) $n = 5$, hexaheptaprenyl pyrophosphate synthase (H-HPP) $n = 3$, and an empty vector control (C) $n = 20$.

Through the first year of growth, plants of the 2002 planting showed trends similar to the plants of the 2001 planting, with the plants actively growing from April to October (Fig. 3). As seen in the 2001 planting, no significant growth differences were observed among any of the N6-5 or AZ-2 transformants and their empty vector controls (data not shown). AZ 101 FPP was significantly larger than the empty vector control as seen in the 2001 planting data, but was not significantly larger than the non-transformed AZ 101 (Fig. 3). The subsequent growth data from this field plot will be analyzed at a later date, as the field is not scheduled for harvest until March 2004.

3.2. Biomass yield

Fresh weight of the plants harvested from the 2001 planting ranged from 0.2 to 8.5 kg, with a mean weight of 2.3 kg. However, no significant differences occurred between transformants and their empty vector controls or among transformants for all lines. Dry weight ranged from 0.1 to 3.9 kg, with a mean weight of 1.0 kg, with no significant differences between any transformants

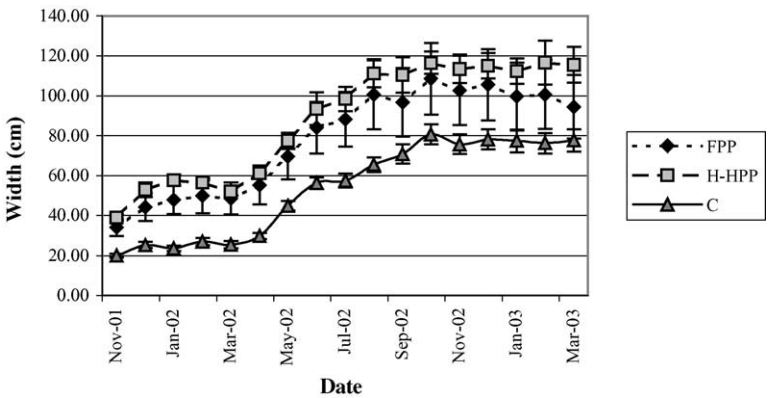

Fig. 2. Mean width (±S.E.) of transgenic guayule (AZ 101) from November 2001 through March 2003. The transgenes included are farnesyl pyrophosphate synthase (FPP) $n = 5$, hexaheptaprenyl pyrophosphate synthase (H-HPP) $n = 3$, and an empty vector control (C) $n = 20$.

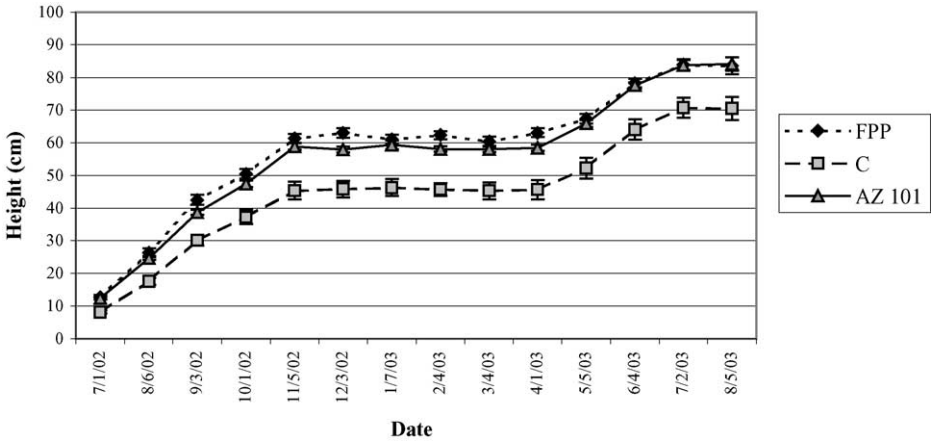

Fig. 3. Mean height (±S.E.) of transgenic guayule (AZ 101) from July 2002 through August 2003. The transgenes included are farnesyl pyrophosphate synthase (FPP) $n = 37$, and empty vector control (C) $n = 16$, and non-transformed AZ 101 $n = 20$.

and their empty vector controls. The only significant difference among transformants was between transformants of different breeding lines: N6-5 H-HPP and AZ 101 H-HPP, with mean dry weights of 0.9 and 2.3 kg, respectively.

3.3. Resin production

A significant increase in resin concentration was present for each gene and empty vector control in the plants from May to January, but there was no significant change in resin concentration, due to any one gene, between January and March (Table 1). In general, plants transformed with FPP had the highest resin concentration at all sample points and plants with either FPP or GGPP had significantly higher resin concentration than the empty vector control in both January and March (Table 1). The N6-5 GGPP and N6-5 H-HPP were not different from the N6-5 empty vector control at any of the sample points. However, N6-5 FPP had a higher resin concentration than N6-5 empty vector control at the final harvest (Table 2). No differences were observed between AZ-2 FPP and the AZ-2 empty vector control at any of the sample points (Table 2). Both AZ 101 FPP and AZ 101 H-HPP, in contrast, had significantly higher resin concentration than the AZ 101 empty vector control throughout the year (Table 2). Varieties that had been transformed with the same gene were compared as well. For those plants carrying only the empty vector, AZ-2 had the highest percent resin during all four months, almost doubling the resin concentration between May and March (Table 2). For those plants transformed with FPP, N6-5 FPP had the lowest resin concentration in May and March, with AZ 101 FPP having a significantly higher resin concentration than N6-5 FPP in those two months (Table 2). The effect of GGPP could not be compared among lines because the only successful transformation was into N6-5, but an increase in resin concentration occurred from approximately 4% in May to around 6% in March (Table 2). A consistent difference existed between the N6-5 and AZ 101 plants transformed with H-HPP, which was significantly higher in AZ 101 H-HPP at all sample dates with the exception of January (Table 2). AZ 101 H-HPP was the only transformant to have a significant increase in resin concentration between January and March (Table 2).

When resin yield was calculated on a per plant basis, AZ 101 FPP and AZ 101 H-HPP produced significantly more resin than the AZ 101 empty vector control (Fig. 4). Neither the AZ-2 nor the N6-5 transformants had greater resin yield than their empty vector controls (Fig. 4). Among transformants of different lines, both AZ 101 FPP and AZ 101 H-HPP had greater resin yields than N6-5 FPP, N6-5 GGPP and N6-5 H-HPP,

Table 1
Resin content of transgenic guayule by gene during the second year of growth (% resin ± standard error)

May 2002	September 2002	January 2003[a]	March 2003[a]
Empty vector control ($n = 27$)			
4.53 ± 0.10 b[b] B[c]	5.28 ± 0.15 abB	6.46 ± 0.14 cA	6.06 ± 0.13 cA
FPP[d] ($n = 16$)			
5.26 ± 0.28 aB	5.69 ± 0.15 aB	7.20 ± 0.19 abA	7.42 ± 0.21 aA
GGPP[e] ($n = 16$)			
4.37 ± 0.16 bB	5.66 ± 0.16 bB	6.59 ± 0.10 aA	6.16 ± 0.10 aA
H-HPP[f] ($n = 71$)			
4.73 ± 0.09 bC	5.59 ± 0.08 bD	6.13 ± 0.09 bcB	5.91 ± 0.11 bA

[a] Data $\log_{10}$ transformed for analysis.
[b] Numbers with the same lower case letter within a column are not significantly different according to Tukey's HSD; $P < 0.05$.
[c] Numbers with the same upper case letter within a row are not significantly different according to Tukey's HSD; $P < 0.05$.
[d] Farnesyl pyrophosphate synthase.
[e] Geranylgeranyl pyrophosphate synthase.
[f] Hexaheptaprenyl pyrophosphate synthase.

whereas only AZ 101 H-HPP had greater resin yield than AZ-2 FPP (Fig. 4).

3.4. Rubber production

The change in rubber concentration throughout the year was very similar for all genes, with rubber concentration being lowest in September and highest in March (Table 3). For all genes and the empty vector, with the exception of the plants transformed by GGPP, there was a significant increase in rubber concentration between January and March 2003 (Table 3). In January, plants transformed with GGPP had a significantly higher rubber concentration than plants transformed with FPP (Table 3). No transformant had a significantly different rubber concentration than its empty vector control

Table 2
Resin content of transgenic guayule by line and gene during second year of growth (% resin ± standard error)

Line	May 2002	September 2002	January 2003[a]	March 2003[a]
Empty vector control				
AZ 101 ($n = 20$)	4.44 ± 0.11 c[b]	5.09 ± 0.14 d	6.20 ± 0.12 cd	5.85 ± 0.07 de
G7-11 ($n = 2$)	4.80 ± 0.29 bc	7.01 ± 0.31 ab	8.08 ± 0.60 ab	8.02 ± 0.41 ab
N6-5 ($n = 5$)	4.77 ± 0.29 c	5.33 ± 0.24 cd	6.84 ± 0.17 bc	6.11 ± 0.12 cde
FPP[c]				
AZ 101 ($n = 5$)	6.33 ± 0.43 ab	6.22 ± 0.30 bc	7.70 ± 0.52 ab	8.31 ± 0.34 a
G7-11 ($n = 6$)	5.02 ± 0.35 c	5.42 ± 0.11 cd	7.09 ± 0.08 ab	7.12 ± 0.28 b
N6-5 ($n = 5$)	4.47 ± 0.34 c	5.48 ± 0.25 cd	6.84 ± 0.21 bc	6.91 ± 0.08 bc
GGPP[d]				
N6-5 ($n = 16$)	4.37 ± 0.16 c	5.66 ± 0.16 cd	6.59 ± 0.10 bc	6.16 ± 0.10 cde
H-HPP[e]				
AZ 101 ($n = 3$)	7.74 ± 0.50 a	7.70 ± 0.54 a	8.81 ± 0.59 a	9.63 ± 0.88 a
N6-5 ($n = 68$)	4.59 ± 0.05 c	5.50 ± 0.06 cd	6.01 ± 0.05 d	5.74 ± 0.05 e

[a] Data $\log_{10}$ transformed for analysis.
[b] Numbers with the same letter within a column are not significantly different according to Tukey's HSD; $P < 0.05$.
[c] Farnesyl pyrophosphate synthase.
[d] Geranylgeranyl pyrophosphate synthase.
[e] Hexaheptaprenyl pyrophosphate synthase.

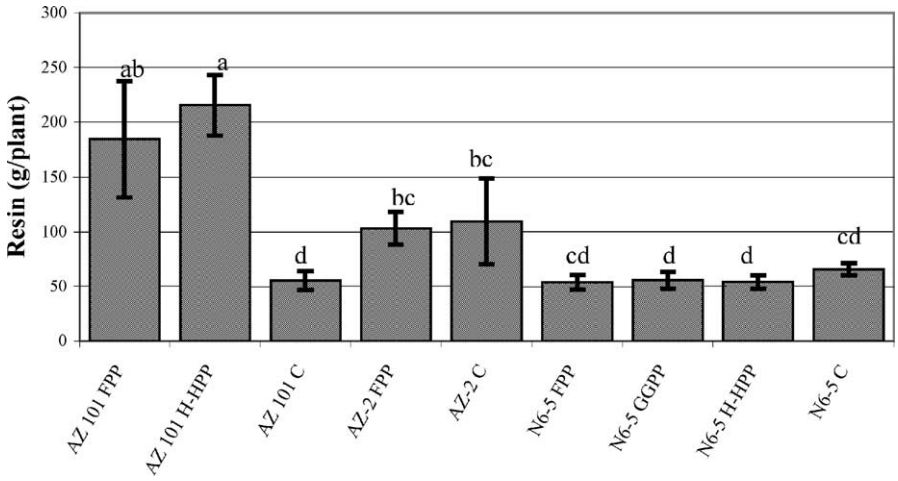

Fig. 4. Mean resin yield (g) per plant of transgenic guayule. Three lines, AZ 101, AZ-2 and N6-5, were transformed with farnesyl pyrophosphate synthase (FPP) $n = 16$, geranylgeranyl pyrophosphate synthase (GGPP) $n = 16$, hexaheptaprenyl pyrophosphate synthase (H-HPP) n = 71 or an empty vector control (C) $n = 27$. Resin yield was $\log_{10}$ transformed for analysis.

(Table 4). However, some differences were present between transformants of different lines. N6-5 GGPP had a higher rubber concentration in January than both AZ 101 FPP and AZ-2 FPP (Table 4), which is consistent with the results found when comparing the overall effect of FPP and GGPP (Table 3). Most of the N6-5 H-HPP plants had higher rubber concentration than AZ 101 FPP and AZ-2 FPP in January and than AZ-2 FPP in March (Table 4). N6-5 FPP also had a significantly higher rubber concentration than AZ 101 FPP and AZ-2 FPP in March (Table 4). When rubber yield was calculated on a per plant basis, no differences were observed between any of the transformants and their controls. Also, no differences were present in rubber yield among any of the transformants, which ranged from 3.7 to 106.3 g per plant.

4. Discussion

The purpose of this study was to evaluate the effect of FPP, GGPP, and H-HPP on the growth and secondary

Table 3
Rubber content of transgenic guayule by gene during the second year of growth (% ± standard error)

May 2002	September 2002[a]	January 2003	March 2003
Empty vector control ($n = 27$)			
2.19 ± 0.18 C[b]	1.58 ± 0.07 B	2.66 ± 0.10 A	3.86 ± 0.09 b[c] A
FPP[d] ($n = 16$)			
1.86 ± 0.23 B	1.30 ± 0.09 B	2.11 ± 0.14 A	3.36 ± 0.22 abA
GGPP[e] ($n = 16$)			
2.00 ± 0.31 C	1.47 ± 0.07 B	3.23 ± 0.19 A	4.06 ± 0.10 abAB
H-HPP[f] ($n = 71$)			
2.31 ± 0.10 C	1.61 ± 0.05 B	3.12 ± 0.07 A	3.86 ± 0.06 aAB

[a] Data natural log transformed for data analysis.
[b] Numbers with the same upper case letter within a row are not significantly different according to Tukey's HSD; $P < 0.05$.
[c] Numbers with the same lower case letter within a column are not significantly different according to Tukey's HSD; $P < 0.05$.
[d] Farnesyl pyrophosphate synthase.
[e] Geranylgeranyl pyrophosphate synthase.
[f] Hexaheptaprenyl pyrophosphate synthase.

Table 4
Rubber content of transgenic guayule by line and gene during the second year of growth (% ± standard error)

Line	May 2002	September 2002[a]	January 2003	March 2003
Empty vector control				
AZ 101 ($n = 20$)	2.24 ± 0.22	1.64 ± 0.08 a[b]	2.68 ± 0.10 bc	3.90 ± 0.10 ab
G7-11 ($n = 2$)	0.99 ± 0.05	1.23 ± 0.10 ab	1.83 ± 0.06 cd	3.31 ± 0.39 a-d
N6-5 ($n = 5$)	2.49 ± 0.19	1.50 ± 0.21 ab	2.90 ± 0.31 abc	3.89 ± 0.14 abc
FPP[c]				
AZ 101 ($n = 5$)	2.71 ± 0.42	1.27 ± 0.48 ab	2.02 ± 0.20 cd	3.10 ± 0.25 cd
G7-11 ($n = 6$)	1.22 ± 0.08	1.12 ± 0.15 b	1.69 ± 0.09 d	2.70 ± 0.14 d
N6-5 ($n = 5$)	1.77 ± 0.37	1.56 ± 0.30 ab	2.70 ± 0.24 a-d	4.42 ± 0.26 a
GGPP[d]				
N6-5 ($n = 16$)	2.00 ± 0.31	1.47 ± 0.07 ab	3.23 ± 0.19 ab	4.06 ± 0.10 ab
H-HPP[e]				
AZ 101 ($n = 3$)	3.61 ± 0.46	1.16 ± 0.20 ab	2.07 ± 0.07 cd	3.21 ± 0.12 bcd
N6-5 ($n = 68$)	2.26 ± 0.09	1.63 ± 0.05 a	3.16 ± 0.07 a	3.89 ± 0.06 ab

[a] Data natural log transformed for data analysis.
[b] Numbers with the same letter within a column are not significantly different according to Tukey's HSD; $P < 0.05$.
[c] Farnesyl pyrophosphate synthase.
[d] Geranylgeranyl pyrophosphate synthase.
[e] Hexaheptaprenyl pyrophosphate synthase.

compound production in guayule. The ultimate goal of the transformations was to increase rubber yield in the transgenic plants by increasing the availability of the initiators for rubber synthesis. These three genes are also precursors to many other compounds in the complex terpenoid pathway. Growth and resin production were measured to determine whether these genes had any effect on other parts of the terpenoid pathway.

In the AZ-2 and N6-5 transformants, there appeared to be no effect of the genes on growth. Any differences that were exhibited among the transformants of these two varieties was probably a varietal effect and not due to the presence of the genes. There appeared to be some effect of FPP and H-HPP on growth in AZ 101. The exceptionally large plants from these transformations indicated a positive effect on growth. However, the results were not conclusive without a non-transformed AZ 101 check. In the 2002 planting, this was rectified and no unusual boost to growth was found when AZ 101 FPP was compared with the non-transformed AZ 101. It may be that during the transformation process the empty vector inserted into an area in the genome that negatively affected growth in AZ 101. Between the two sequential yearly field plantings, there were five separate empty vector transformations of AZ 101 (four in 2001 and one in 2002) that all exhibited the same reduction in growth, and were not significantly different from each other. Therefore, it is possible that a decrease in growth is not due to where the plasmid inserted, but an antagonistic effect of what was carried on the plasmid, which was cancelled out by the genes carried by AZ 101 FPP and AZ 101 H-HPP.

Interestingly, there appeared to be an effect of transformation on resin production from May to January, but not rubber production. Both of the AZ 101 transformants, AZ 101 FPP and AZ 101 H-HPP, had high resin yields per plant. This could be accounted for in two of different ways. The first is higher overall biomass in the plant. The transformants were large, but statistically there was no difference in biomass compared with the empty vector control. However, this may have been due to the small number of plants within each transformation, such that a very small or very large plant within any transformation would have a very large effect on the variability, obscuring any significant biological differences. The other possible explanation for the relatively high resin yield is a higher resin concentration within the transformants. Both AZ 101 FPP and AZ 101 H-HPP had much higher resin concentration than not only their empty vector control but also all of the other transformants as well (Table 2). This high resin concentration could be an indication that FPP and H-HPP are

positively affecting resin production in AZ 101, but no definitive conclusion can be made until analysis of the data from the 2002 planting, which also had a negative control.

Little or no effect of the transgenes is apparent on rubber production (Tables 3 and 4). The only difference was that N6-5 FPP had a higher rubber concentration than AZ-2 FPP and AZ 101 FPP (Table 4). The higher rubber concentration in N6-5 FPP could be a line effect due to their relatively small size compared with AZ-2 FPP and AZ 101 FPP, whose parent lines were selected for size (Thompson and Ray, 1989; Ray et al., 1999). Size differences could also affect rubber yield by diluting rubber in the larger plants that would be expressed by a lower rubber concentration, but similar rubber yields as smaller plants with a higher rubber concentration.

Although the rubber concentration and rubber yield data indicate that there was no effect of these three genes on rubber production, other possible alternative explanations for this lack of difference can be considered. High initiator concentrations, as are probably produced in the transgenics due to their enhanced endogenous prenyl transferase activity (unpublished results), would enhance the production of new rubber molecules, but an overall increase in the amount of rubber would only occur at nonlimiting IPP concentrations (Cornish 2001; Cornish and Scott, 2004). The low affinity of the guayule rubber transferase for IPP ensures that rubber can only be made when IPP is not in demand by other metabolic processes. Thus, enhancing the production of IPP in guayule may have a stronger effect on rubber production. Alternatively, the initiators may have rapidly been metabolized by the resin biosynthetic pathway. The remarkably strong affinity of the rubber transferase for the initiators suggests that bypassing rubber biosynthesis is unlikely and that substrate limitations by IPP are the most likely cause of the unaltered rubber levels. Due to apomictic reproduction, altering guayule according to traditional breeding techniques has been difficult. Transgenic technology may give breeders the tools they need to increase rubber synthesis significantly. Although the initial round of transformations increased resin content without impacting rubber levels, it did show that transformation is possible and should be a practical approach once the rubber transferase gene is cloned.

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